**PATENT**
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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| Applicant: | H. Robert Horvitz et al. | Art Unit: | 1643 |
| Serial No.: | 09/087,136 | Examiner: | Karen Canella |
| Filed: | May 28, 1998 | Customer No.: | 21559 |
| Title: | TUMOR SUPPRESSOR PATHWAY IN *C. ELEGANS* | | |

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION OF DR. H. ROBERT HORVITZ UNDER 37 C.F.R. § 1.132 TRAVERSING GROUNDS OF REJECTION

Under 37 C.F.R. §1.132, I declare:

1. I am an inventor of the subject matter described and claimed in the above-captioned patent application.

2. I have read the Office Action mailed on March 26, 2002.

3. *lin-37* is useful as an agent for the modulation of proliferation. This is evidenced by the fact that members of the *C. elegans* Class B synMuv gene pathway, to which *lin-37* belongs, function in a conserved tumor suppressor pathway, and that *C. elegans* Class B synMuv genes function as negative regulators of the evolutionarily conserved Ras signal transduction pathway.

4. *C. elegans* Class B synMuv genes constitute a conserved tumor suppressor pathway. This is supported by the fact that many *C. elegans* synMuv genes have homology to mammalian genes known to be involved in cancer. For example, *lin-35*, a *C. elegans* class B synMuv gene, encodes a close homolog of mammalian tumor suppressor protein Rb. In humans, mutations in Rb promote tumor formation, most commonly, retinoblastoma. The mammalian homolog of *lin-53*, another *C. elegans* class B synMuv gene, encodes Rb-binding protein, p48 (72% identity). In addition, mammalian DP and E2F, homologs of *C. elegans* synMuv genes LIN-55 and E2F-1, function together to regulate the transcription of genes essential for cell cycle progression.

5. The *C. elegans* Class B synMuv genes, to which *lin-37* belongs, antagonize the Ras signal transduction pathway. The Ras pathway is conserved throughout evolution. In a variety of organisms, from *C. elegans* through humans, Ras regulates cell proliferation. For example, Ras activating mutations have been found in many human tumors. The importance of the Ras pathway in human oncogenesis is emphasized by the fact that Ras mutation frequency is among the highest of any gene associated with human cancer (Hunter et al., submitted with Reply to Office Action, mailed November 6, 2001).

In *C. elegans,* Ras signalling controls vulval cell proliferation. Mutations in *C. elegans* synMuv genes result in a synthetic multivulva phenotype, characterized by excess vulval cell proliferation. Because the Ras pathway is highly conserved, insights gained into the regulation of *C. elegans* Ras will likely have important implications for the regulation of human Ras. It is likely that additional mammalian homologs of *C.*

*elegans* synMuv genes will be identified, and that these genes will function in a mammalian tumor suppressor pathway that regulates Ras signalling.

6. *C. elegans lin-37* is a Class B synMuv gene for which no mammalian homolog has yet been identified. In the nematode, *lin-37* antagonizes Ras signaling and functions in a tumor suppressor pathway to regulate vulval cell proliferation. Mammalian homologs of *lin-37* will likely antagonize Ras signaling and function in a mammalian tumor suppressor pathway to regulate cellular proliferation. Thus *lin-37* and its mammalian homologs are likely to be useful in the treatment of a proliferative disorder (e.g., cancer).

7. As further evidence that synMuv genes (e.g., *lin-37*) are useful for the treatment of a proliferative disorder, such as cancer, I submit herewith the following references: Saito et al., "Malignant Worms: What Cancer Research Can Learn from *C. elegans*," *Cancer Investigation*, 20:264-275, 2002 (Exhibit A); and Chang et al., "*C. elegans* Vulval Development as A Model System to Study the Cancer Biology of EGFR Signaling," *Cancer and Metastasis Reviews* 18:203-213, 1999 (Exhibit B); each of these references was written by a third party expert, and each accepts that mammalian homologs of *C. elegans* synMuv genes likely function in oncogenesis, and thus may be useful for the treatment of proliferative disorders, such as cancer.

8. Saito et al., provides a review of the usefulness of *C. elegans* in cancer research (page 264, first column third paragraph to second column).

> The purpose of this review is to explain the use of the model organism, *C. elegans*, and its relevance for cancer research. We discuss important contributions in three areas. First, homologs of human oncogenes and tumor suppressors have been

> found to act in genetic pathways that control well defined biological processes in *C. elegans*. Importantly, the gene networks elucidated in *C. elegans* appear widespread in the animal kindom and usually are similar to those used in humans. Thus, significant insights have been obtained into the function of human cancer genes by studying their counterparts in the nematode.

With respect to vulval cell fates, at page 270, first column, second and third paragraphs, and second column, first paragraph, Saito states:

> [T]he finding that the *C. elegans* Ras homolog functions in a growth-factor receptor signaling pathway that controls cell fate was an enormously important contribution to the studies of the *ras* oncogene.
>
> Many other genes have been found to regulate the *ras* signal-transduction pathway. Although their discussion goes beyond the scope [of] this review, it is important to mention that several of these genes are presently novel but will likely have mammalian counterparts. Thus, as soon as such mammalian genes are identified a candidate function is already available. Some of these genes may have roles in carcinogenesis. (Emphasis added.)

Clearly, Saito accepts that *C. elegans* genes that regulate Ras signal transduction, as *lin-37* does, are likely to play a role in mammalian cancer. Furthermore, with respect to our work on the synMuv genes (Lu et al., *Cell* 95:981-991, 1998), Saito states, at page 271, first column, line 6,

> [T]he *lin-35* class B product is similar to the retinoblastoma-susceptibility protein pRb. In addition, another class B gene, *lin-53*, encodes a protein similar to RbAp48 that interacts with pRb. It will be of great interest to determine whether homologs of the other synMuv genes act as tumor-suppressor genes in humans.

Clearly, Saito accepts that mammalian homologs of *C. elegans* synMuv genes are likely to function in mammalian cell proliferation.

9. Chang provides a review of the importance of *C. elegans* vulval development research in defining evolutionarily conserved signal transduction pathways for the study of oncogenesis. Chang states:

> Molecular genetic studies of *C. elegans* vulval development have helped to define an evolutionarily conserved signaling pathway from an EGF-like ligand through EGF receptor, Ras and MAP kinase to the nucleus. Further studies have identified novel positive regulators such as KSR-1 and SUR-8/SOC-2 and negative regulators such as cbl/SLI-1. The many negative regulatory proteins might serve to prevent inappropriate signaling, and thus are analogous to tumor suppressor genes. ...(Page 203, Abstract). (Emphasis added.)

And, at page 206, first column, third paragraph.

> Yet another set of apparently redundant negative regulators are known. Genetically, there are two classes of the so-called synthetic multivulva (synMuv) genes, class A and class B.

Chang therefore also accepts that mammalian homologs of *C. elegans* synMuv genes, which negatively regulate Ras signaling, are likely to act as mammalian tumor suppressor genes. Such genes would likely be useful for the treatment of a proliferative disorder, such as cancer.

10. As yet another line of evidence indicating that the synMuv genes (e.g., *lin-37*) are considered to encode tumor suppressors useful in the treatment of a proliferative disorder, I submit Lu *et al.* (*Cell* 95:981-991, 1998) (Exhibit C), a paper that I published with a member of my lab. In this paper, we propose that mammalian homologs of synMuv genes act in a tumor suppressor pathway (at page 987, second column, second paragraph, to page 988).

> *lin-35* encodes a protein related to Rb, and *lin-53* encodes a protein with striking similarity to an Rb-binding protein, p48 (72% identity). *lin-35*, *lin-53*, and a *C. elegans* histone deacetylase gene act in the same genetic pathway to antagonize a Ras signal transduction pathway in *C. elegans*. We propose that in mammals Rb, p48, and histone deacetylase genes act in a tumor suppressor pathway that involves mechanisms and molecules similar to those of the synMuv pathway in *C. elegans* and that may well antagonize a mammalian Ras pathway.

This work was peer-reviewed by top scientists and published in *Cell*, suggesting that our reviewers accepted that *C. elegans* synMuv genes, such as *lin-37*, are useful in altering cell proliferation.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: July 8, 2002

Dr. H. Robert Horvitz